

## Reports

### *Ethambutol\* Changes the Color Coding of Carp Retinal Ganglion Cells Reversibly*

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The influence of ethambutol on retinal function was studied by recording ganglion cell responses in isolated carp retinas superfused with a Ringer solution containing different concentrations of ethambutol (0 mg/liter, 10 mg/liter, 20 mg/liter, 30 mg/liter). The results indicate that ethambutol reversibly affects color opponency, without changing the sensitivity of the underlying receptor processes. The amacrine and bipolar cells are the most likely candidates to be affected by ethambutol. *Invest Ophthalmol Vis Sci* 24:128-133, 1983

Visual function is affected in about 0.5 to 1.5% of patients treated with ethambutol, a commonly used tuberculostatic drug.<sup>1</sup> These patients show nontypical color vision defects. For example, in the Farnsworth (100 Hue) test errors are present along both the deutan and protan axes.<sup>2,3</sup> The visual acuity of some of these patients decreases, although generally without visual field losses.<sup>4</sup> After treatment is stopped, pretreatment vision is generally regained.<sup>5</sup>

The site where ethambutol affects the visual system is not clear. Very high doses of 1300-1600 mg/kg/day (about 65× the therapeutic dose) cause demyelination of the optic nerve in rhesus monkey (*Macaca Mulatta*).<sup>6</sup> Ophthalmoscopic examination has revealed toxic effects in the human retina.<sup>7</sup>

Zrenner and Krüger<sup>3</sup> have performed an extensive psychophysical and electrophysiological study on two affected patients. Since both patients showed normal ERGs and since the signals of all three cone types were present in the visual evoked potential (VEP), they concluded that these toxic effects do not manifest themselves in a loss of a particular receptor mechanism. They attributed the visual defects caused by ethambutol to a modification of color-opponent neural mechanisms.

If this modification of color-opponent mechanisms occurs at the retinal level, then recording the ganglion cell activity changes caused by ethambutol will be a more direct approach than the ERG, which is determined predominantly by receptors and Müller cells.

In the present study the influence of ethambutol

on the ganglion cell responses was examined in isolated carp retina. Carp has three cone types, with action spectra peaking in the blue (around 450 nm), the green (around 530 nm), and the red (around 620 nm) regions of the visual spectrum.<sup>8</sup> In teleost fish color interactions occur as distal as the horizontal cells,<sup>9-11</sup> and most ganglion cells show spatial as well as spectral opponency.<sup>12,13</sup> It will be demonstrated, that spectral opponency can be modified reversibly, when ethambutol doses in the range of 10-30 mg/liter are applied.

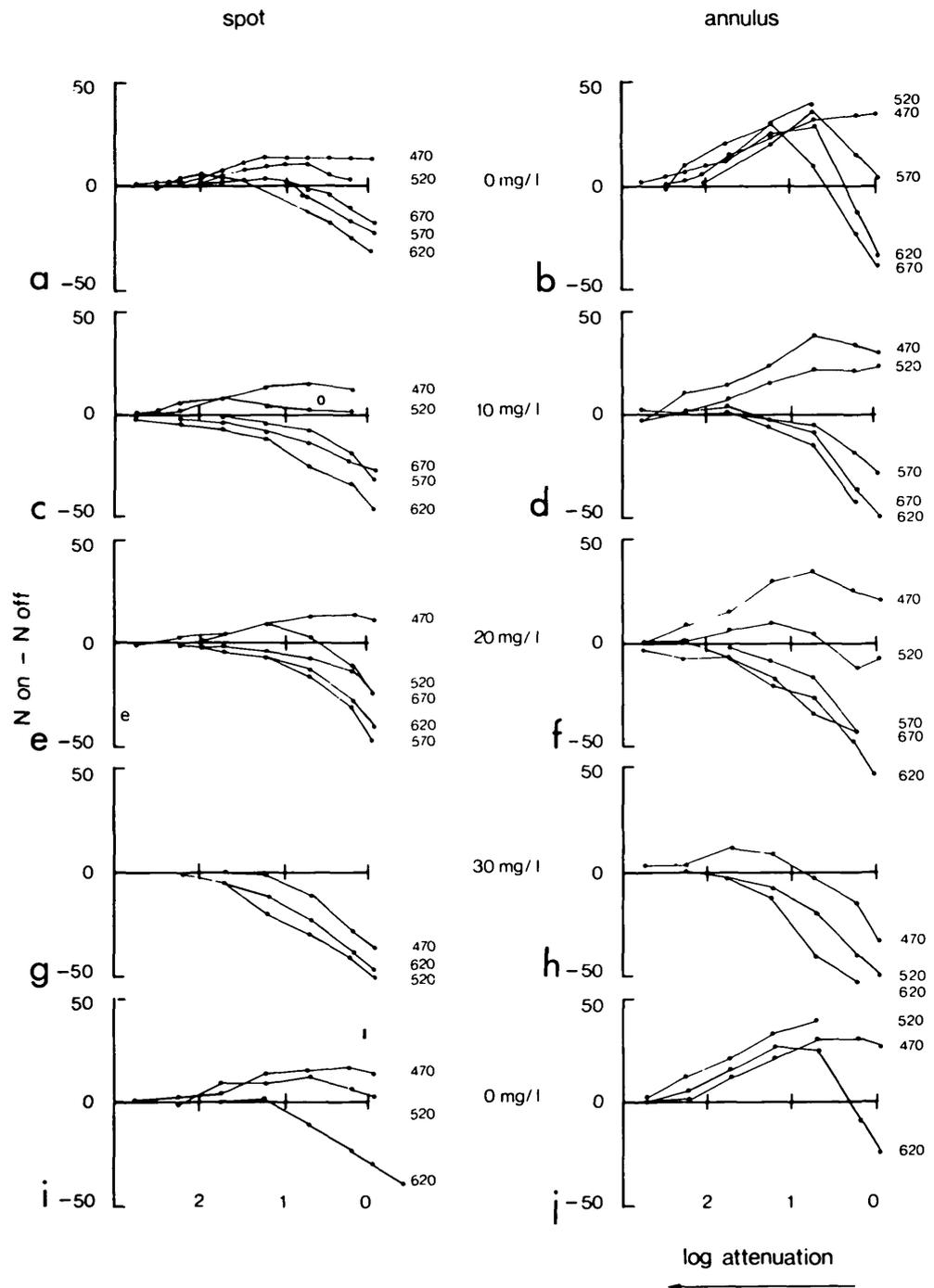
**Materials and Methods.** Normal carp (*Cyprinus carpio*) of 600 to 800 g were enucleated in the dark. The retina was isolated and placed receptor side up in a preparation chamber filled with a standard Ringer solution (NaCl: 120 mM; KCl: 3.5 mM; CaCl<sub>2</sub>: 1.6 mM; NaHCO<sub>3</sub>: 22.6 mM; MgSO<sub>4</sub>: 1.6 mM; dextrose: 10 mM; a mixture of 97.5% O<sub>2</sub> and 2.5% CO<sub>2</sub> is pumped through this solution to maintain a pH of 7.4). This solution is pumped through the chamber exchanging the medium with a time constant of .8 minute. Temperature is kept at 17.5 C. After each change in the composition of the Ringer solution from addition of ethambutol, the retina was kept in the dark or at a constant level of illumination for at least 20 min, before data collection was restarted.

A two-channel optical stimulator could project spots and annuli of different diameters on the retina from underneath. Wavelengths in the range of 410-780 nm could be set in one beam with a monochromator (width at half amplitude 20 nm) and in the other beam with interference filters (width at half amplitude 10 nm). The intensity of the 450W Xenon lamp could be attenuated with neutral density wedges. The narrow band light stimuli are modulated by shutters that interrupt the beams once per 2 sec with a duty cycle of 50%. To restrict rod intrusion and to maintain a constant state of adaptation the entire retina is illuminated continuously by a 509 nm background light of low photopic intensity ( $5 \cdot 10^{14} \text{ Q} \cdot \text{s}^{-1} \cdot \text{m}^{-2}$ ).

Ganglion cell responses were recorded with glass-coated PtIr electrodes that penetrated the retina from above. In this condition the stimulus does not create a shadow of the electrode on the retina. Spikes were

\* Myambutol is manufactured by Lederle, Division of American Cyanamid Co., Wayne, NJ.

**Fig. 1.** Response vs log intensity curves recorded from a carp ganglion cell with red, green and blue sensitive cone inputs. Left column response to spot stimulation (diameter: 0.30 mm). Right column response to annular stimulation (inner diameter: 2.20 mm; outer diameter: 3.30 mm). Duty cycle of the stimuli: 800 msec on, 800 msec off. Ethambutol concentration was changed in steps of 10 mg/liter. Figures I and J show the results obtained upon return to the standard Ringer solution. After each concentration change, the retina was kept at least 20 min in the dark to settle to the new medium. The vertical axis is the difference between the average number of spikes recorded in successive ON phases and OFF phases of the stimulus. The horizontal axis is the log attenuation of the stimulus light intensity. Twenty stimulus cycles were averaged. Zero log units attenuation corresponds with  $9 \cdot 10^{16} \text{ Q} \cdot \text{s}^{-1} \cdot \text{m}^{-2}$ .



shaped and played over a loudspeaker. The spikes were counted during the ON phase and OFF phase of the stimulus, and these numbers were recorded on a two-channel chart recorder. Criterion response spectra were obtained by setting the intensity attenuation in such a way that a just audible change in the spike discharge could be heard. Stable recordings from isolated ganglion cell were possible for up to 11 hours.

**Results.** Changes in the response properties of 14 spectral opponent ganglion cells were recorded. Ethambutol had similar effects on all these units. Figures 1A and B show the number of spikes vs log intensity curves elicited by spot (left column) and annular (right column) stimulation of different wavelengths. In this figure the average difference between the number of spikes recorded during ON and OFF phases of the stimulus is plotted.

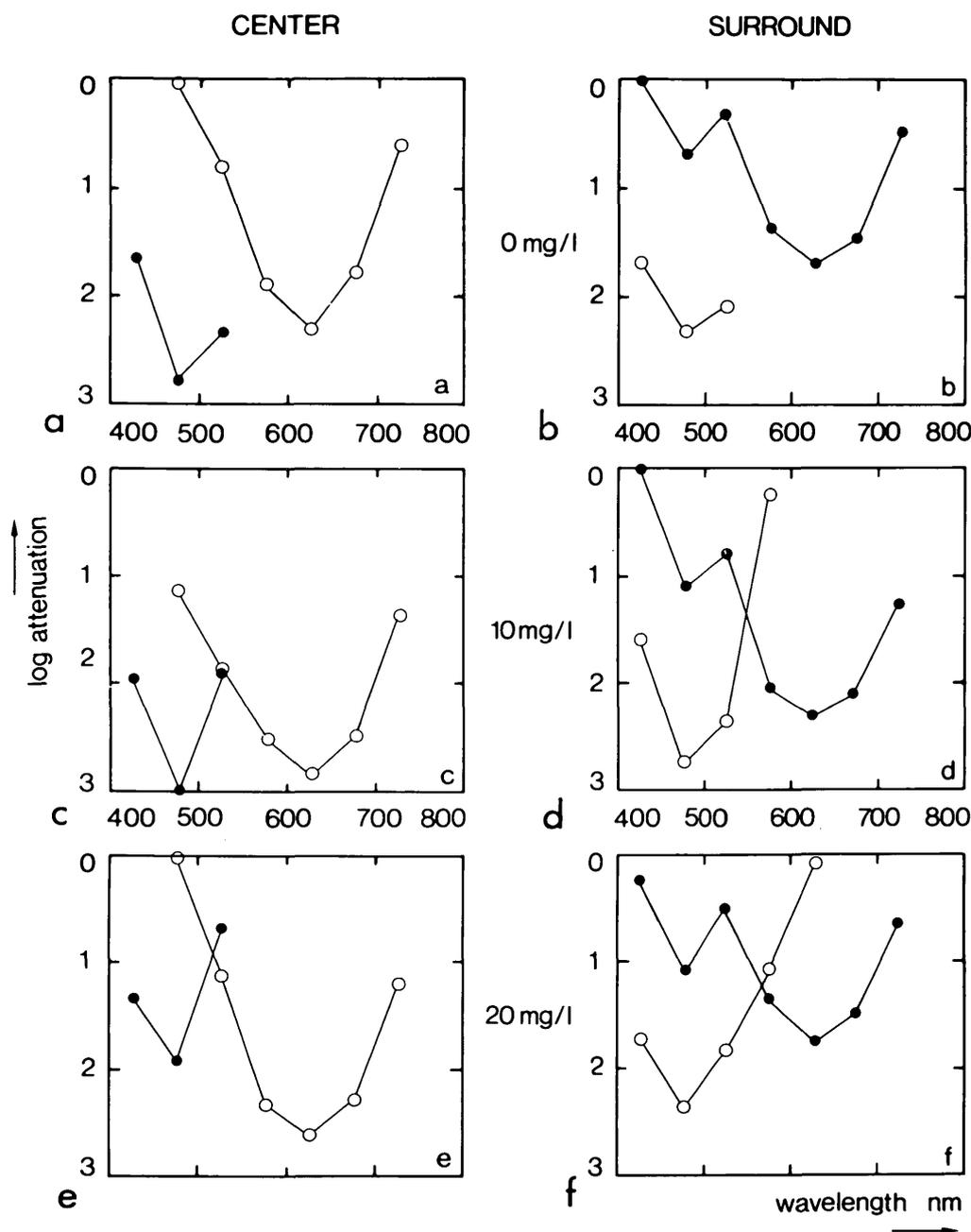
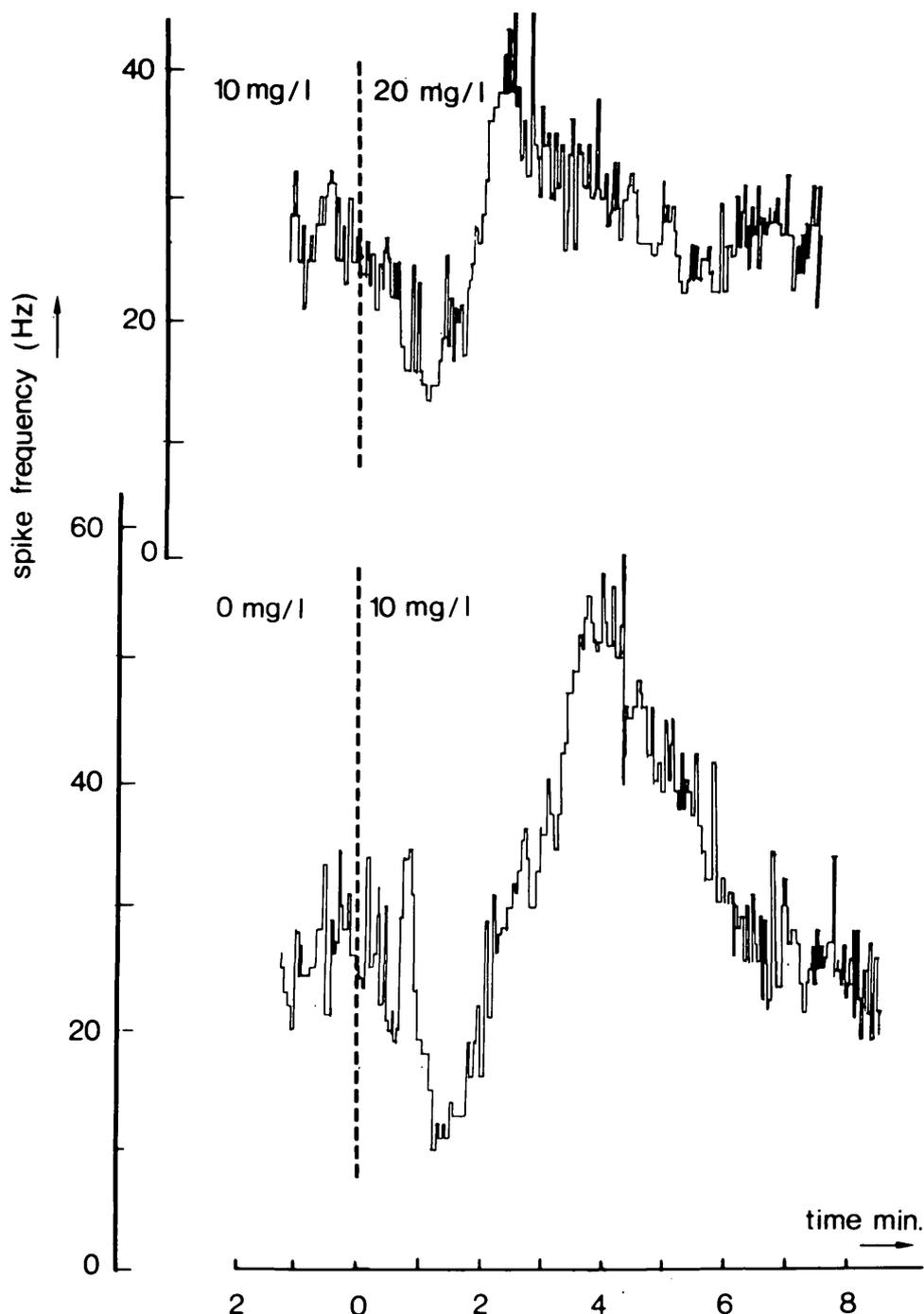


Fig. 2. Criterion response spectra for a center red ON, green OFF (left column); surround red OFF green ON (right column) ganglion cell, when different concentrations of ethambutol are added to the Ringer solution. The center stimulus was a spot with diameter 0.15 mm; the surround stimulus was an annulus with inner diameter 1.80 mm and outer diameter 3.30 mm. Duty cycle of the stimulus was: 1 sec on, 1 sec off. The log attenuation that yielded the criterion response is plotted against the wavelength of the stimulus. Thresholds were reproducible within 0.15 log units.

Since this unit received input from all three cone types (with different signs) the response vs log intensity curves have a rather complicated shape. One of the consequences of this is that a constant response criterion like a just noticeable change in the spike discharge does not result in unambiguous spectral characteristics. For this reason the effect of ethambutol is shown in the form of response vs log intensity curves. Data were recorded at least 20 min after the replacement of the initial solution by another one with a new ethambutol concentration. Inspection of Figure 1 shows that application of ethambutol affects the responses to spot stimulation in the same way as to annular stimulation.

For increasing ethambutol concentrations the color antagonism becomes less pronounced. For example, the responses obtained in the 10 mg/liter medium still have opposite signs in the short (ON response) and long (OFF response) wavelength regions of the visible spectrum, whereas only OFF responses are obtained in the 30 mg/liter medium. This change in color antagonism cannot be attributed to variation in absolute sensitivity of underlying receptor mechanisms since for each wavelength the lowest intensity yielding a response is not affected.

After 11 hours of recording from the same unit the standard Ringer solution was replaced, and the response vs log intensity curves depicted in Figures 11



**Fig. 3.** Spike density profiles of the maintained activity from the unit of Figure 2 upon adding 10 mg/liter ethambutol. The number of spikes counted during successive (1 sec) time intervals is plotted vs time. The dashed line marks the start of the concentration change, in the lower profile from 0 mg/liter to 10 mg/liter, in the upper profile from 10 mg/liter to 20 mg/liter. Replacement of the Ringer solution occurs with a time constant of 0.8 min.

and J were recorded. Comparison of these figures with Figures 1A and B shows that the unit had recovered fully to the initial state.

The spontaneous activity from the same unit was also measured. No changes with concentration of ethambutol could be established.

Figure 2 shows criterion response spectra recorded from a ganglion cell with the most frequently encountered spectral and spatial coding in carp retina: antagonistic coding for red and green in both center and surround. The coding of this particular unit was:

center red on and green off (left column), surround red off and green on (right column). As was the case for the unit presented in Figure 1, the effect of ethambutol expresses itself on the interaction between the cone mechanisms and not on their absolute sensitivities. This is most evident in the surround responses of Figure 2.

Normally the presence of a strong red response inhibits the other color mechanisms' contribution to the spike discharge. For example, the green process in the surround (Fig. 2B) can only be found for stim-

ulus wavelengths up to 525 nm with the peak shifted towards shorter wavelengths. In the presence of ethambutol, however, this inhibition weakens: at 10 mg/liter ON responses can be recorded up to 575 nm (Fig. 2D); at 20 mg/liter up to 625 nm (Fig. 2F). Since this change is achieved without significant changes in the sensitivities of the two receptor mechanisms, the functional site of the effect must be an active color opponent mechanism.

This unit also recovered completely upon return to the standard Ringer solution.

Figure 3 shows the spontaneous activity of the ganglion cell of Figure 2 upon adding 10 mg/liter ethambutol. Directly following the ethambutol application (taking in account the 0.8 min time constant) dramatic changes occur. At first the spontaneous activity is severely reduced. Next the spike frequency increases to about twice the initial rate. Finally the firing pattern slowly returns to the original rate, and after about 5 min the spontaneous activity is back to the original mean level with, however, somewhat larger variations.

**Discussion.** The most prominent effect of ethambutol on ganglion cell function in the carp retina is that it affects the color opponent characteristics of the ganglion cell responses. These changes are not secondary to a change of the spatial organization, nor to a change in the absolute sensitivities of the underlying receptor mechanisms, nor to a change in the maintained discharge of the ganglion cells. The effects of ethambutol on the color opponent interactions are reversible.

Several authors<sup>2,14-17</sup> have suggested that ethambutol causes demyelination of the optic nerve. However, this is quite unlikely since affected patients often have abnormal color vision without visual field or acuity losses that are generally associated with demyelination.

The results of this study clearly show that ethambutol affects visual function in the retina. Dick et al<sup>18</sup> have shown that in the mudpuppy retina ethanol enhances ON responses of bipolars and the b-wave of the ERG, while suppressing OFF responses. Since ethambutol is like ethanol, an alcohol, their action on the retina might be related. However, in our experiments ethambutol did not show a selective action on center or surround neither on ON or OFF mechanisms. The changes monitored in ganglion cell responses indicate that the most prominent effect of ethambutol is that inhibitive interactions between opponent color mechanisms (active interactions) are weakened. When ethambutol is applied it appears as if the receptor processes feed independently into the ganglion cells, as such responses of all contributing mechanisms can be recorded in the yellow-green re-

gion of the spectrum. A similar change can also account for the observed color deficiencies in affected patients. On the basis of our results it is possible to speculate about the retinal cell types that are affected by ETHAMBUTOL.

Since Zrenner and Krüger<sup>3</sup> did not find abnormal ERGs and could measure signals from all three cone types in the VEP it is unlikely that ETHAMBUTOL affects the receptors. The present study confirms this since no changes were found in the absolute sensitivities of the cone mechanisms that underly the ganglion cell responses.

Because no long-term changes in the ganglion cells' maintained activity was observed, it is unlikely that the site of action of ethambutol is proximal to the ganglion cells' dendritic synapses. Toyoda and Kujiraoka<sup>19</sup> have shown that in carp retina polarization of any of the horizontal cell types influences the relative sensitivity of the bipolar surround process and hence the center/surround balance. So it is likely that also at the ganglion cell level this balance will be changed by polarization of the horizontal cells. However, no changes were observed in the center/surround activity balance when ethambutol was applied. This indicates that ethambutol does not affect the horizontal cells. This is supported by Zrenner and Krüger's observation that affected patients have normal ERGs.

Our results, therefore, indicate that the most likely site of ethambutol action is in the inner plexiform layer and that the most likely candidates to be affected by ethambutol are the amacrine and bipolar cells.

**Key words:** acquired color vision defects, ganglion cell responses, ethambutol, color coding, drug effects

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## References

1. Pau H and Wahl M: Myambutol-Schädigung des Auges. *Ber Dtsch Ophthalmol Ges* 72:176, 1972.
2. Trusciewicz D: Farnsworth 100-Hue test in diagnosis of ethambutol-induced damage to optic nerve. *Ophthalmologica* 171:425, 1975.
3. Zrenner E and Krüger CJ: Ethambutol mainly affects the function of red/green opponent neurons. *Doc Ophthalmol Proc Ser* 27:13, 1981.
4. Carr RE and Henkind P: Ocular manifestations of ethambutol. Toxic amblyopia after administration of an experimental antituberculous drug. *Arch Ophthalmol* 67:566, 1962.

5. Pahlitzsch H and Tiburtius H: Augenuntersuchungen bei Behandlung mit dem neuen Tuberkulostatikum Ethambutoldihydrochlorid Lederle (Myambutol). *Klin Monatsbl Augenheilkd* 154:228, 1969.
6. Schmidt IG: Central Nervous System effects of ethambutol in monkeys. *Ann NY Acad Sci* 135:759, 1966.
7. Gross V, Eule H, and Hager G: Auswertung einer Toxizitätsstudie bei intermittierender Ethambutol-Medikation. *Klin Monatsbl Augenheilkd* 163:17, 1973.
8. Tomita T, Kaneko A, Murakami M, and Pautler EL: Spectral response curves of single cones in the carp. *Vision Res* 7:519, 1967.
9. Svaetichin G: Spectral response curves from single cones. *Acta Physiol Scand Suppl* 134:17, 1956.
10. Spekrijse H and Norton AL: The dynamic characteristics of color-coded S-potentials. *J Gen Physiol* 56:1, 1970.
11. Stell WK, Lightfoot DO, Wheeler TG, and Leeper MF: Goldfish retina: Functional polarization of cone-horizontal cell dendrites and synapses. *Science* 190:989, 1975.
12. Wagner HG, MacNicol EF Jr, and Wolbarsht ML: The response properties of single ganglion cells in the goldfish retina. *J Gen Physiol* 43:45, 1960.
13. Spekrijse H, Mooy JEM, and Van den Berg TJTP: Photopigments and carp ganglion cell action spectra. *Vision Res* 21:1601, 1981.
14. Weder W: Myambutolschäden des Sehnerves. *Ber Dtsch Ophthalmol Ges* 72:172, 1972.
15. Stärk N: Toxische Sehnervenschädigung durch Myambutol. *Med Klin* 67:913, 1972.
16. Orou F, Sideroff G, and Schabel F: Frequenzuntersuchungen von Optikuskrankungen im Rahmen der Myambutol-Behandlung. *Klin Monatsbl Augenheilkd* 161:601, 1972.
17. Thaler A, Heilig P, Heiss WD, and Lessel MR: Toxische Schädigung des Nervus opticus durch Ethambutol. *Klin Monatsbl Augenheilkd* 165:660, 1974.
18. Dick E, Miller RF, and Dacheux RF: Neuronal origin of B- and D-waves in the I-type ERG. *ARVO Abstracts. Invest Ophthalmol Vis Sci* 19(Suppl):34, 1979.
19. Toyoda J and Kujiraoka T: Analyses of bipolar cell responses elicited by polarization at horizontal cells. *J Gen Physiol* 79:131, 1982.

## *Asteroid Bodies—An Ultrastructural Study*

Hedva Miller, Benjamin Miller, Hanna Rabinowitz, Shlomo Zonis, and Izhak Nir

An ultrastructural study of asteroid bodies in a vitreous aspirate from a patient suffering visual loss as a result of asteroid hyalosis is presented. Under appropriate conditions of fixation and high resolution transmission electron microscopy, a lamellar arrangement with a periodicity of 4.6 nm was observed. This lamellar arrangement is typical of liquid crystalline phases of lipids in water. X-ray microanalysis confirmed the presence of calcium and phosphorus in the asteroid bodies. We propose that the asteroid bodies are not true crystals but rather liquid crystals of phospholipids in the vitreous humor. *Invest Ophthalmol Vis Sci* 24:133–136, 1983

Asteroid bodies are seen as brilliant particles floating in an apparently normal vitreous body. They usually appear unilaterally in the 60–80 age bracket. Their presence does not cause any impairment in vision<sup>1</sup> except in very rare instances.<sup>2</sup>

Even though the asteroid bodies have been quite extensively investigated,<sup>1,3–5</sup> their composition is still not fully elucidated, and there are various hypotheses concerning their origin, organization and mode of formation.<sup>1,3,4</sup>

The asteroid bodies are known to be composed of lipids, calcium, and phosphorus.<sup>1,3,5</sup> Feldman<sup>6</sup> showed that the lipids are mainly sphingomyelin with some smaller amounts of ceramide dihexoside, cerebroside, and cholesterol.

Lipids in water are known to form a lamellar liquid crystalline phase.<sup>7</sup> Such lamellar arrangement can be revealed by transmission electron microscopy only when appropriate conditions of fixation are being used.<sup>8</sup>

In an attempt to elucidate further the structural organization of asteroid bodies we carried out ultrastructural and x-ray elemental analysis on asteroid bodies from a vitreous aspirate. By using appropriate conditions of fixation required for the preservation of the lamellar arrangement in liquid crystalline phases, we were able to demonstrate such an arrangement in the asteroid bodies.

**Materials and Methods.** *Case report:* A 70-year-old patient was referred to our clinic because of gradual bilateral loss of vision. His best-corrected visual acuity was 20/100 in both eyes due to densely filled vitreous by typical asteroid bodies. He had some minimal posterior capsular lens opacities, in both eyes, that could only slightly attribute to his visual loss. Fundi, as far as they could be seen, were normal. As the visual acuity over a follow-up period of a year dropped to 20/140, we performed a pars plana lens-ectomy and vitrectomy on his left eye, using the Ocutome System®. Nine months later his best-corrected vision, in the left eye, was only 20/60, and that because he had developed cystoid macular edema.

*Preparation for transmission electron microscopy*